

In re application of:	W. Marasco, et al.	Group No.:	1632
Serial No.:	08/822,033	Examiner:	J. Woitach
Filed:	March 24, 1997		
For:	NUCLEIC ACID DELIVERY SYSTEM, METHOD OF SYNTHESIS AND USES THEREOF		

REMARKS

Claims 1, 3 – 5, and 7 – 16 were rejected under 35 U.S.C. §103(a) as being unpatentable over Beug et al. in view of Chaudhary et al. and Wu et al.

Applicants respectfully submit that this rejection should be withdrawn for the following reasons.

The present invention is directed to a nucleic acid delivery system that specifically delivers the nucleic acid to a specific cell type, and to a method for selectively delivering a nucleic acid to a specific cell type. This is accomplished by taking advantage of the ability of antibodies to bind to different cell surface proteins and thus discriminate between cell types. In the present invention, the antibody is fused to a nucleic acid binding protein such as protamine; the protamine in turn binds the nucleic acid that is delivered to the cell.

It is undisputed that there is no anticipation of this application by the prior art cited by the Examiner. As explained below and also in the Declaration of Dr. Marasco (copy attached hereto), the present invention is not obvious because the combination of references in no way suggests that a recombinant fusion protein would deliver nucleic acid more precisely than a chemical conjugate.

Beug discloses a system for targeted nucleic acid delivery that focuses exclusively on the use of transferrin to target specific cell types, not an antibody. The Examiner has cited Wu as teaching that “an antibody provides an effective means for specifically targeting a fusion protein to a particular epitope on the surface of a desired cell.” Moreover, Wu, like Beug, is primarily directed to a specific ligand, not antibodies. Indeed, the *only* mention of antibodies is the single statement referenced by the Examiner, which reads “other ligands such as antibodies or polypeptide hormones, may also be employed” (col. 6, lines 5 – 7). However, the law is clear that references must be read in their entirety. Wu teaches the use of “ligands” to target specific cell types, and exemplifies the claimed invention using asialoglycoprotein to target hepatocytes. Beug focuses on transferrin. Reading these references in their entirety, even including a single reference to other strategies, would in no way teach the skilled artisan to use an antibody fusion

protein approach. The combination in no way teaches that antibodies have any special advantage to confer. This is particularly clear because Wu lumps antibodies together with peptide hormones, which have a completely different mechanism of action. Thus, contrary to the Examiner's position, both Wu and Beug do not teach that an antibody is an effective means to target a fusion protein.

The Examiner has also contended that the addition Chaudhary teaches the use of a fusion protein for selectively targeting a nucleic acid to a cell. Applicants respectfully disagree. Chaudhary was studying delivery of proteins, namely immunotoxins, to cells, not nucleic acid. Furthermore, Chaudhary was directed to an improved cloning technique. The skilled artisan who was interested in delivering nucleic acid to a cell would not have looked to Chaudhary, because its focus was so different.

The Examiner further contends that these references suggest making a recombinant fusion protein, but both Beug and Wu, which are directed to nucleic acid delivery, only make chemical conjugates. A passing reference in Beug to the possibility of a fusion protein must be contrasted with Wu's teaching against such a construct. Wu actually teaches *against* the combination, by indicating that it was beneficial that the covalent bond of the chemical conjugate could be cleaved once it had been taken up by a cell. See Marasco Declaration, ¶ 11; Wu et al., Col. 5, lines 37 – 48. By contrast, the purpose of the Chaudhary fusion protein was to deliver targeted toxins for cytotoxic therapy, to kill certain cells. For such an application, it would not matter if the peptide bond was cleaved following uptake. However, for anyone interested in selectively delivering a nucleic acid to a cell, Wu teaches against a fusion protein approach, because of the difficulty of cleaving a peptide bond intracellularly.

Moreover, Beug also teaches against such a fusion protein construct in stating that different ratios of transferrin to a nucleic acid binding protein can be used, including 10:1 to as low as 1:4. A fusion protein approach does not allow such flexibility, instead requiring a rigid 1:1 ratio. Using a fusion protein does not allow the advantages of a chemical conjugate according to Beug, for achieving different molar ratios. Thus, Wu points out a chemical

conjugate permits a better bond and Beug teaches that the chemical conjugate provides greater flexibility in the ratio of materials that can be used.

Accordingly, the combination actually teaches the skilled artisan to use a ligand – not an antibody – chemically conjugated to the nucleic acid binding moiety – not a fusion protein.

Finally, the combination in no way suggests the unexpected specificity achieved with the fusion protein. As explained in detail in the attached Declaration of Wayne A. Marasco (¶¶ 15 – 21), applicants' surprising discovery was that their antibody fusion protein approach confers greater specificity than the chemical conjugate approach. This advantage is clearly illustrated in the applicants' paper, Li et al., *Cancer Gene Ther.* 8: 555-565 (2001), previously submitted.

The Examiner has contended that the results reported in Li et al. are no different from those described in Chaudhary and Wu. Chaudhary and Wu used ligands, not antibodies, and applicants' comparison is antibody to antibody. The relevant comparison is between the two approaches, fusion protein v. chemical conjugate, rather than between the use of either approach for selective and non-selective cells. Only Li et al. directly compares the two approaches, and its result is clear: unexpectedly, the fusion protein affords greater selectivity than the chemical conjugate approach.

Figure 6C of Li et al. shows the results for the fusion protein approach to selective nucleic acid delivery. By comparing gene transfer in SKBR3 (an Erb B2⁺ cell) with MCF7 (an Erb B2⁻ cell), it was found that the uptake and gene expression of the reporter nucleic acid was approximately 8 – 10 fold higher in the Erb B2⁺ cell than in the Erb B2⁻ cell. Figure 7B shows the analogous results for the chemical conjugate approach to selective nucleic acid delivery, using the same components as used in Figure 6 C. In these experiments, however, the selectivity is only about 4 – 5 fold higher. Thus, when the two approaches were compared head to head, the unexpected result was the two types of delivery system do not behave the same.

The Examiner has argued that the “focus” of each of the prior art references was selective targeting. The chemical conjugate approach as shown by Li is selective, but it is not nearly as selective as the fusion protein. Applicants were the only group to employ an antibody fusion

protein strategy for selective delivery of a nucleic acid, and they have now demonstrated its surprising effectiveness. The Examiner has pointed to a statement in the discussion section of Li that indicates that there is room for improvement of the present method, and used this statement to argue that the Li publication does not focus on the advantages of the increased selectivity of the fusion protein approach. However, the cited paragraph is discussing the overall efficiency of gene transfer, which is not the subject of the claims. *Efficient* processes for gene transfer were already known at the time of the present invention; instead, what was sought was a method for *selective* delivery. That is precisely what the present invention provides, as stated further in the same paragraph: "As far as the [chemical conjugate] DPSL is concerned ... it is **not as selective** as compared with the [fusion protein] ScFv-P-S..." [emphasis added] Thus, although there may be room for improving the efficiency of the fusion protein approach, it is clear that it achieves its objective of conferring greater selectivity in targeting specific cells. That specificity is the focus of the application. Such improved selectivity with a recombinant fusion protein is in no way suggested by the prior art references, which disclose chemical conjugates for targeted nucleic acid delivery.

Thus, the rejection of the claims should be withdrawn.

Claim 6 stands rejected under 35 U.S.C. §103(a) as being unpatentable over Beug et al. in view of Chaudhary et al. and Wu et al as applied to Claims 1, 3 – 5, and 7 – 16, and further in view of Ryder et al.

Applicants respectfully submit that this rejection should be withdrawn for the following reasons.

Applicants respectfully submit that the addition of Ryder et al. to the combination in no way overcomes the essential deficiency of the references discussed above. As indicated by the Examiner, Ryder discloses sequence specific binding of Jun to a nucleic acid. Ryder in no way discloses targeted delivery of nucleic acids to cells, nor provides any motivation to the skilled artisan. Thus, Ryder cannot cure the fundamental defect in the original combination of references, which do not teach the use of an antibody fusion protein for selective delivery of a

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nucleic acid. Accordingly, for the reasons of record which are repeated herein, and for the reasons mentioned above, this rejection of the claims should also be withdrawn.

In view of the foregoing, applicants respectfully submit all claims are in condition for allowance. Early and favorable action is requested.

Respectfully submitted,

Date: _____

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Customer No. 26248

Ronald I. Eisenstein
Reg. No. 30,628
NIXON PEABODY LLP
101 Federal Street
Boston, MA 02110
(617) 345-6054